Food Chemistry 278 (2019) 665–673

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Solid dispersion-based spray-drying improves solubility and mitigates beany flavour of pea protein isolate

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ARTICLE INFO

Keywords:
Plant protein
Pea protein isolate
Spray-drying
Solid dispersion
Gum arabic
Maltodextrin
Solubility
Beany flavour

ABSTRACT

Recently, there is a strong interest in incorporation of pea protein as a preferred alternative to animal protein into protein-fortified food. However, the utilization of pea protein as a food ingredient has been largely limited because of its poor functionality. The aim of this study was to understand if solid dispersion-based spray-drying processing could be applied to enhance the solubility and mitigate off-flavour in pea protein isolate (PPI). The influence of amorphous matrix carrier type (gum arabic and maltodextrin), and PPI to carrier ratio (90:10–60:40) on physical properties, solubility, and off-flavour profile of PPI was investigated. The results demonstrated the possible mechanism by which factors such as surface area/volume ratio, hydrogen bonding, and electrostatic interaction between PPI and carriers enhance PPI solubility. Meanwhile, beany flavours were mitigated through the unfolding of secondary structure of PPI by forming solid dispersions with gum arabic or maltodextrin during spray-drying.

1. Introduction

In recent years, there is a growing interest in using pulse seed proteins as meat alternatives and functional ingredients in food products. For instance, pea protein, a common pulse protein extracted from yellow split pea (*Pisum sativum* L.), has attracted a great deal of attention due to its gluten-free feature, high nutritional value, cheap price and great sustainability (Adebiyi & Aluko, 2011; Lam, Can Karaca, Tyler, & Nickerson, 2018). One particular interest of pea protein applications in food industry is pea protein-fortified beverages, including protein shakes, sports drinks, and protein juice blends.

However, pea protein is still largely underutilized as a functional ingredient in food systems. The major reason limiting the utilization of pea protein is its poor functional properties, such as low solubility and unpleasant beany and rancid flavours. Solubility is an important prerequisite for a protein to be an effective nutritional ingredient in high moisture food applications, such as protein fortified beverages. In terms of pea protein isolate (PPI), its unique subunits and secondary conformation structures have been reported to affect, not only the solubility and beany flavour, but also the *in vivo* digestibility and availability of essential amino acids (Carbonaro, Maselli, & Nucara, 2015). Pea protein isolate mainly consists of salt-soluble globulin (60–80%) with the subunits of legumin (11S) and vicillin (7S), along with water-soluble albumin (2S, 15–25%) (Shand, Ya, Pietrasik, & Wanasundara, 2007). Moreover, a higher content of β-sheet and a relatively low amount of α-helix are present in the secondary structure of PPI (Carbonaro et al., 2015). The high amount of globulin with a predominant β-sheet structure determines the lower solubility of PPI which directly contributes to the chalky mouthfeel of PPI-fortified protein beverages.

More importantly, earthy/green (beany flavour) and grassy/painty (rancid flavour) flavours formed during pea storage and pea protein extraction narrow their application in food systems, and primarily affect the enjoyment of foods (Arora & Damodaran, 2010). In general, earthy and grassy flavours are produced by the activation of endogenous lipoxygenase that directly catalyzes the oxidation of unsaturated fatty acids in pulse seeds during storage (Jiang et al., 2016). The primary oxidation products, hydroperoxides, produced by enzymatic oxidation, can further breakdown to form small molecules of beany or rancid volatiles, such as alcohols, aldehydes, ketones, acids and lactones (Jiang et al., 2016). It has also been reported that off-flavour compounds have a tendency to tightly bond with the main mass of pea protein through covalent bonds, hydrogen bonding, or hydrophobic interactions during dry or wet protein isolation processes (Arora & Damodaran, 2010). Thus, the bonded beany and rancid flavour molecules in dry pea protein products will be carried over to the food products in which an undesirable off-flavour will be perceived upon consumption (Jiang et al., 2016). Numerous studies have attempted to

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https://doi.org/10.1016/j.foodchem.2018.11.074
Received 28 August 2018; Received in revised form 27 October 2018; Accepted 13 November 2018
Available online 14 November 2018
0308-8146/

identify and reduce beany flavour in plant protein products, e.g. solvent treatment, thermal processing, and fermentation (Arora & Damodaran, 2010; Lv, Song, Li, Wu, & Guo, 2011). Nevertheless, those treatments invariably result in protein denaturation and a loss of solubility, although some off-flavours can be reduced. Therefore, it is imperative to explore alternative methods that can not only mitigate the off-flavour in pea protein, but also increase the solubility.

Solid dispersion-based spray-drying technique has been reported as a promising means to improve the solubility of poorly water-soluble ingredients (e.g., curcumin and rebaudioside A), and reduce protein bitterness (Patel, Patel, Chakraborty, & Shukla, 2015). The fundamental concept of this technique is the dispersion of poorly water-soluble ingredients in an amorphous matrix carrier through spray-drying that creates a dispersed state with improved solubility (Pang, Ma, Zhang, & He, 2015). Amorphous gum arabic (GA) and maltodextrins (MD) would be the ideal candidates for this purpose as they are commonly used as wall materials for bioactive compound encapsulation or as flavour-masking agents for odour mitigation in spray-drying processing. For instance, the increased stability and dissolution rate of hydrolyzed casein was achieved by using gum arabic as a solid dispersion carrier during spray-drying (Subtil et al., 2014). Therefore, the solid dispersion-based spray-drying technique may be an optimal means to enhance the solubility of PPI.

The purpose of this study was to investigate the influence of an amorphous matrix carrier type (GA or MD) and PPI-carrier ratios on the structural properties and functionalities of PPI processed by solid dispersion-based spray-drying technique. Our hypothesis is that the structural reorientation of poorly soluble PPI can improve its solubility and facilitate the release of strongly bound off-flavour compounds upon the formation of a dispersed state with an amorphous matrix carrier after spray-drying. Therefore, the present investigation was undertaken to (i) characterize the structural and functional properties of spray-dried (SD) PPI-GA or PPI-MD and compare them with physically mixed (PM) PPI-GA and PPI-MD, (ii) assess their solubility and beany flavour profiles and (iii) establish possible relationships between structural changes and functional properties.

2. Materials and methods

2.1. Materials

Yellow pea flour (19% protein) was purchased from Harvest Innovations (Indianola, IA, USA). Gum arabic (GA) (Instantgum™ BA) was kindly donated by Nexira (Somerville, NJ, USA). As reported by the manufacturer, the viscosity of GA in 25% water at 20 °C is 60–100 mPa.s. Spray- dried maltodextrin (MD, DE 18) (Cargill Dry MD™ 01918, Lot No. 11916CHYSA) from partially hydrolyzed corn starch was kindly provided by Cargill (Minneapolis, MN, USA). Other chemicals and reagents used in this study were of analytical grade and purchased from VWR (Chicago, IL, USA). All solutions were prepared using ultrapure distilled de-ionized water (DDW, 18.2 MΩ cm, Barnstead Nanopure ultrapure water system, Thermo Scientific, MA, USA).

2.2. Preparation of pea protein isolates

Freeze dried (FD) pea protein isolate (PPI) was extracted from yellow pea flour, using an alkali extraction-isoelectric precipitated method, as described by Lan, Chen, and Rao (2018) without modification. The triplicated proximate analyses on final PPI powder were carried out according to AOAC methods (2016): 79.50% protein (% N × 6.25, wet basis), 5.28% moisture, 0.77% lipid, 4.61% crude ash, and 9.84% carbohydrate (by difference from 100%).

2.3. Preparation of spray-dried PPI with gum arabic or maltodextrin

In total, eight spray-dried (SD) samples were prepared by the following procedures: the PPI was stirred magnetically in water at ambient temperature for 0.5 h at 500 rpm. Then, the mixed solutions of varying PPI-carrier ratios (90:10, 80:20, 70:30, and 60:40 w/w) were prepared by mixing PPI solution with an equal amount of GA or MD solution using a magnetic stirrer for a further 0.5 h at the same speed. The resulting mixtures were adjusted to pH 7 ± 0.05 using 0.1–1 M HCl, and then were fed into a Büchi mini spray-dryer (Model B-290, New Castle, DE, USA) with a feed rate of 0.15 l/h, aspirator flow of 40 l/h to form spray-dried (SD) samples. The total solid content of all prepared mixtures was fixed at 20 wt%. The inlet and outlet air temperature were maintained at 160 and 100 °C, respectively. In addition, eight physically mixed (PM) samples were prepared by geometric mixing of PPI with GA or MD with the same mixing ratios as described above, using a pestle and mortar. Both spray-dried and physically mixed powders were collected and stored in glass bottles at ambient temperature in a dry place before characterization. The details about the samples formulated by both spray-drying and physical mixing are listed in Table S1 (Supporting Information).

2.4. Scanning electron microscopy (SEM)

A scanning electron microscope (SEM) (JEOL Model JSM-6490LV, Peabody, MA, USA) was used to study the morphological properties of powder samples. A speck of sample was attached to an adhesive carbon tab on a cylindrical aluminium mount and the excess was blown off with a stream of nitrogen gas. Then, the sample was sputter-coated with gold (Cressington 108 auto, Ted Pella, Redding, CA, USA) and examined at 500×, 1000×, 1500× and 3000× magnifications with an accelerating voltage of 15 kV. Selected micrographs (1500×) were obtained as representative of each sample for subsequent analysis.

2.5. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) spectra of all SD and PM samples were obtained, using a Varian FTIR spectrophotometer (CA, USA) equipped with an attenuated total reflectance accessory (Pike Miracle, Pike Technologies, Madison, WI, USA), Globar (MIR) source, KBr beam separator, and MCT detector. The samples were scanned in the absorbance mode from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} at 32 scans. The background was collected before each sample scanning.

2.6. X-ray diffraction (XRD)

X-ray diffraction (XRD) pattern of all SD and PM samples was measured, using an Ultima IV X-ray diffractometer (Rigaku, TA, USA) with Cu-Kα operating at 40 kV and 44 mA. Samples were scanned at 2θ range from 5 to 45° at a rate of 1.2°/min and a step size of 0.05° and the patterns were displayed without any modification.

2.7. Percent protein solubility

The percent protein solubility (PPS) of SD samples, at both pH 4.5 and 7, was measured according to the method described by Lan et al. (2018) without modification. Briefly, the SD or PM sample (fixed at 1 wt% PPI concentration) was stirred magnetically in water at ambient temperature for 5 h before being adjusted to pH 4.5 or 7. Then, the protein concentration of each sample, before and after centrifugation (3220×*g*, 10 min), was determined, using the Bradford dye-binding method. The PPS (%) was calculated as: (protein concentration of supernatant/protein concentration of initial solution) × 100%.

2.8. Characterization of off-flavour compounds

Volatile compounds in SD samples were identified using headspace solid phase micro extraction gas chromatography-mass spectroscopy (HS-SPME-GC–MS) (Agilent 7890B GC system and 5977 MS system, CA, USA) based on the method described by Jiang et al. (2016) with some modifications. Briefly, the powder sample (1.00 g) was first weighed into a 20 ml GC vial with aluminium caps and PTFE/silicone septa. The powder sample was then suspended by adding 2 ml of DDW water and incubated in a water bath at 30 °C for 1 h. The suspended sample in the GC vial was further incubated in an autosampler heating block at 55 °C for 15 min before measurement. Volatiles were extracted and adsorbed for 2 min by SPME fibres (DVB/CAR/PDMS, Supelco Inc., PA, USA). Volatiles were then released from the fibre (250 °C for 3 min) and separated by a capillary column ZB-WAX (60 m × 0.25 mm I.D. × 0.25 μm film thickness, Supelco Inc., PA, USA). Helium carrier gas was used at a constant flow rate of 2 ml/min. The oven temperature was programmed by heating the sample at 5 °C/min from 40 °C (held for 4 min) to 250 °C (held for 20 min). Mass spectra were acquired at an ionization energy of 70 eV with a scan range of 40–350 Da. Compounds were identified using the NIST 14L mass spectral database, and absolute peak areas in area counts of selected beany (1-pentanol and 1-octen-3-ol) and rancid (hexanal) flavour markers were recorded.

2.9. Statistical analysis

The experimental treatments were arranged in a completely randomized design (CRD) with two replicates. All measurements were performed at least twice, using freshly prepared samples, and values were expressed as means ± SD. Significant differences between means ($p < .05$) were identified using one-way analysis of variance (ANOVA) and F-protected LSD by SAS software (Version 9.3, SAS Institute Inc., NC, USA).

3. Results and discussion

3.1. Pea protein surface morphology

SEM images can reveal the shape and the exterior morphology of a sample to a great extent. The morphological characteristics of controls (FD PPI, SD PPI, GA, and MD) and solid dispersion-based spray-dried PPI with different carriers (GA or MD) under variable ratios (G1-4, M1-4) are shown in Fig. 1.

A bulk plate-shaped morphology with a smooth surface was observed in FD PPI powder (Fig. 1), similar to the morphology of other freeze-dried plant proteins, such as rice protein isolate (Zhao, Xiong, Selomulya, & Chen, 2013). Freeze-drying can increase the opportunity of protein molecules to come into close contact with one another, thus forming aggregation after a long time of processing (Gong et al., 2016). In contrast, SD PPI powders showed more uniform particle size with spherical shapes, smooth surfaces, and some concavities. Similar morphologies were observed in spray-dried lentil proteins (Joshi, Adhikari, Aldred, Panozzo, & Kasapis, 2011). The appearance of concavities on the surface of SD PPI powders can be attributed to the rapid evaporation of water droplets during quick spray-drying (Costa et al., 2015; Subtil et al., 2014). Similar to SD PPI, the morphology of carrier (GA or MD) demonstrated typical spherical shapes with dented surfaces, presumably because of the shrinkage of the particles during drying processing. Such morphology of GA and MD has been widely reported by others (Bae & Lee, 2008; Bertolini, Siani, & Grosso, 2001)

However, a substantial surface morphology difference was observed between PPI-GA and PPI-MD samples after solid dispersion-based spray-drying. In particular, G1 (spray-dried PPI-GA at a ratio of 90:10) had a smooth surface with some concavities mingling with a hollow internal structure, a typical characteristic of spray-dried powders highlighted by red arrow (Fig. 1. G1), which suggests the formation of matrix-type microspheres (Bae & Lee, 2008; Subtil et al., 2014). The smooth surface with some concavities was gradually replaced by a rough and wrinkled surface as the content of gum arabic increased from 20 to 40 wt%, becoming closer to the morphology of GA. In contrast, all solid dispersion-based spray-dried PPI-MD samples had greater smooth surface with some concavities, and more agglomerated particles than had spray-dried PPI-GA. The morphology changes, from spherical shapes with some concavities to fragmented and agglomerated particles, was observed with increase of MD content (M2-M4). Such morphological changes of PPI-MD were also reported in other spray-dried systems, such as gelatin-MD, and pea protein concentrate (PPC)-MD systems (Chuaychan & Benjakul, 2016; Pierucci, Andrade, Farina, Pedrosa, & Rocha-Leao, 2007). All the above morphological changes indicated an increased overall surface area to volume ratio (S/V ratio), with more porous, rough and wrinkled surfaces, upon increasing the content of amorphous matrix carrier in the PPI solid dispersion system.

3.2. Fourier transform infrared spectroscopy (FTIR) and pea protein secondary structure

FTIR analysis provides information of functional groups attached to the protein backbone (Pang et al., 2015). In order to better understand whether the solid dispersion of PPI with GA or MD could modify the functional groups of proteins or generate interactions between PPI and carrier after spray-drying, the IR spectra of SD PPI samples (G1-4, M1-4) were recorded in the range 4000–400 cm^{-1} and compared that with the physically mixed (G′1-4, M′1-4) samples at the same ratio (Fig. 2).

From Fig. 2a, the spectrum of PPI showed a strong absorption band at 3275 cm^{-1} (–OH contraction vibrations of hydrogen bonding water), at 2956 and 2926 cm^{-1} (two C—H stretching bands), at 1633 cm^{-1} (C═O stretching vibration from acetylated unit –$CONH_2$, characteristic of amide I group), at 1529 cm^{-1} (N—H bending and C—N stretching combination, feature of amide II), at 1392 cm^{-1} (the overtone vibration of N—H bending and C—N stretching, feature of amide III), at 1155 cm^{-1} (C—O—C symmetric stretching band), and at 1063 cm^{-1} (C—O strong vibration stretching). These results were supported by previous reports on the spectrum of pea protein (Costa et al., 2015; Gonzalez-Martinez et al., 2017; Huang, Sun, Xiao, & Yang, 2012; Wang et al., 2006). Regarding carriers, the spectrum of GA exhibited typical absorption bands of polysaccharide at 3272 cm^{-1} (–OH broad band), at 2914 cm^{-1} (C—H stretching vibration), at 1597 and 1412 cm^{-1} ($–COO^-$ asymmetric and symmetric stretching vibration, respectively), and at 1014 cm^{-1} (C—O—C stretching vibration) (Fig. 2a). The same functional groups of MD were also registered at 3275, 2917, 1639, 1410, and 1012 cm^{-1} (Fig. 2c). These findings were in agreement with previous reports (Gonzalez-Martinez et al., 2017; Gulao, de Souza, Andrade, & Garcia-Rojas, 2016; Santiago-Adame et al., 2015).

In general, the spectrum of SD PPI-carriers (GA or MD) was quite similar to that of the parent PPI as no appreciable peak disappearance or wavenumber shifts was observed, except the proportionally weaker intensity of absorption band as the ratio of the carrier (GA or MD) increased. This is due to the smaller quantities of PPI in solid dispersion-based spray-dried samples than in the parent form (Fig. 2a & c). In addition, the absorption bands of SD PPI-GA samples in the frequency range 3200–3500 cm^{-1} became gradually wider as the PPI-GA ratio changed from 90:10 (G1) to 60:40 (G4). This band is generally assigned to O—H stretching of inter- or intra- molecular interactions, indicating that hydrogen bonding plays a major role in molecular interaction between PPI and GA (Hinterstoisser & Salmen, 1999). Besides, the absorption band of SD PPI-GA in the range of the amide I band (at 1633 cm^{-1}), amide II band (at 1529 cm^{-1}), and amide III group (at 1392 cm^{-1}) decreased drastically as compared to that of parent PPI (Fig. 2a). This can be explained by the possible electrostatic interaction between the amino groups of PPI and the carboxyl groups of GA which mainly occurred in the carbonyl-amide region (Gonzalez-Martinez et al., 2017). This is supported by a negligible reduction of these

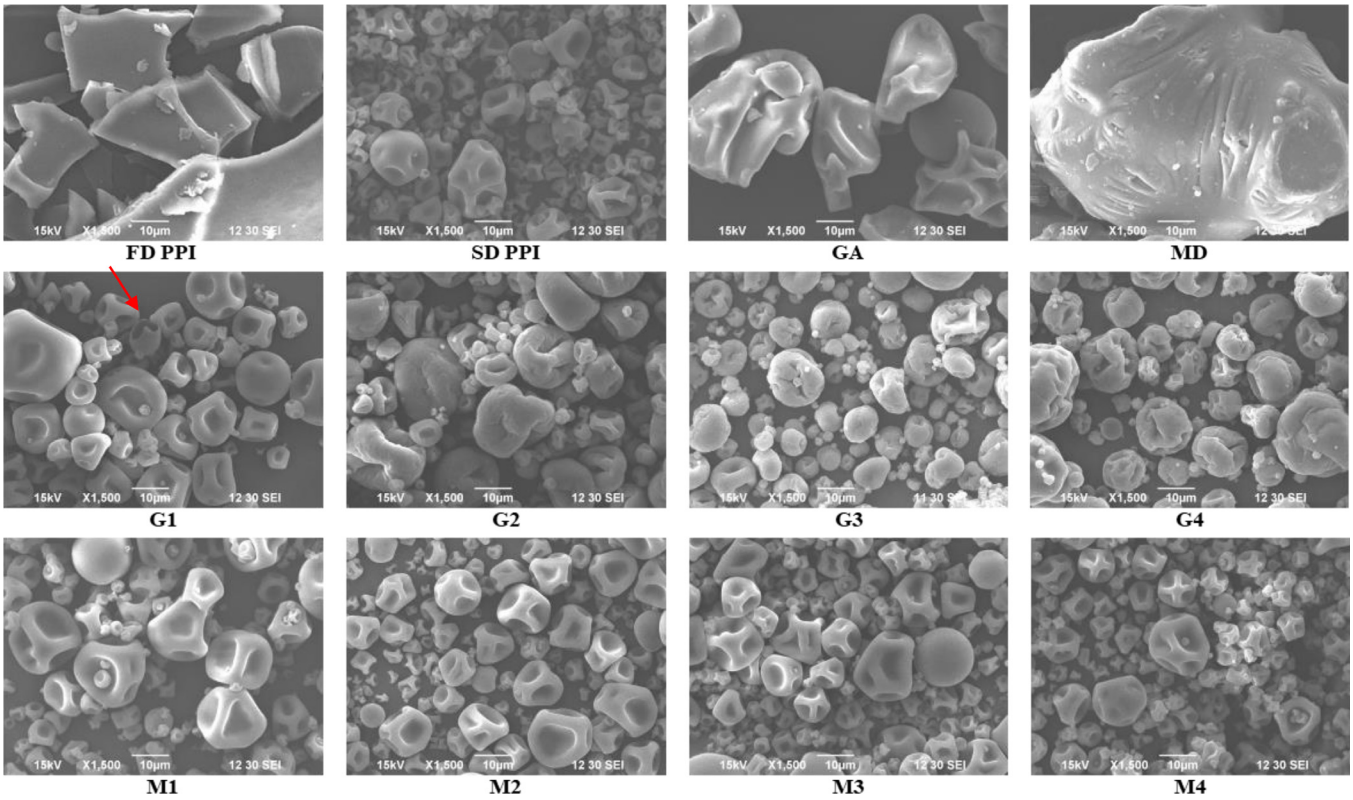

Fig. 1. SEM images of freeze-dried pea protein isolate (FD PPI), spray-dried pea protein isolate (SD PPI), gum arabic (GA), maltodextrin (MD), spray-dried PPI-GA at ratios of 90:10 (G1), 80:20 (G2), 70:30 (G3), and 60:40 (G4) and spray-dried PPI-MD at ratios of 90:10 (M1), 80:20 (M2), 70:30 (M3) and 60:40 (M4).

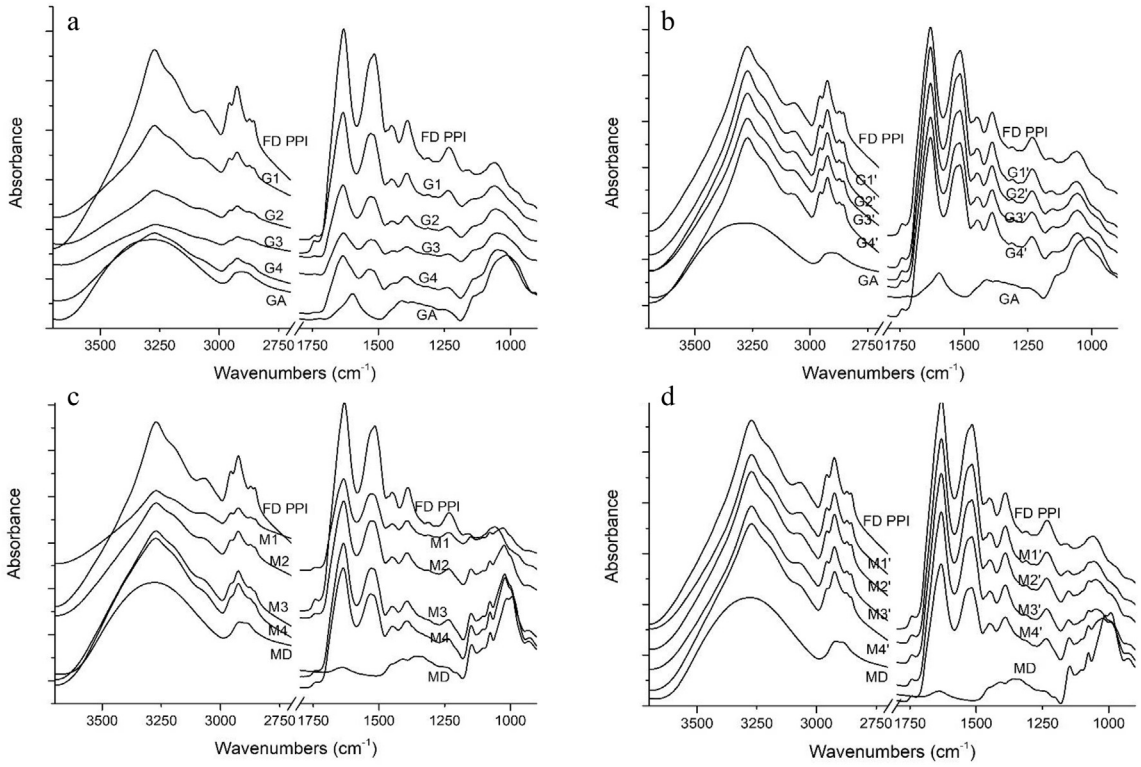

Fig. 2. FTIR spectra of controls and spray-dried samples. The patterns of (a) FD PPI, GA, and spray-dried G1-G4, (b) physically mixed G1′-G4′, (c) MD, and spray-dried M1-M4, (d) physically mixed M1′-M4′.

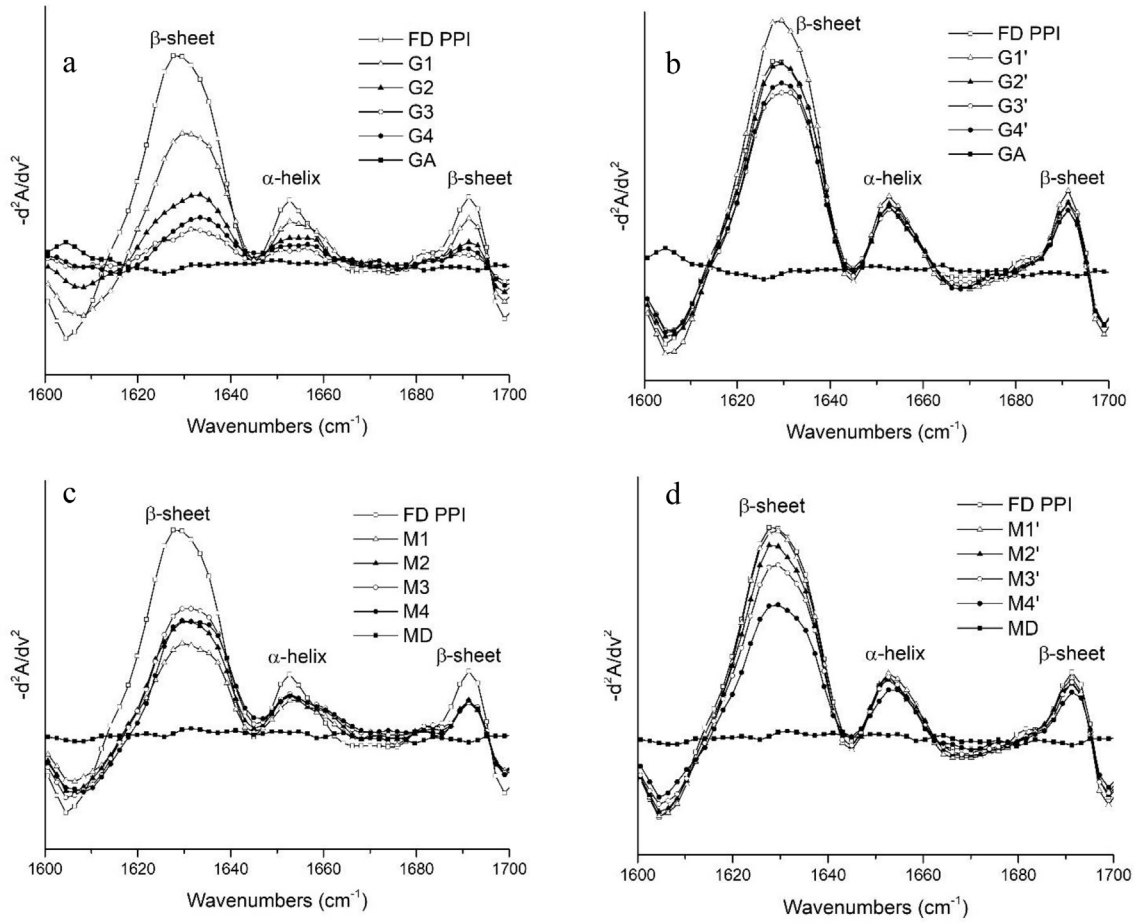

Fig. 3. Second derivative of FTIR spectra of controls and spray-dried samples. The patterns of (a) FD-PPI, GA, and spray-dried G1-G4, (b) physically mixed G1′-G4′, (c) MD and spray-dried M1-M4 and (d) physically mixed M1′-M4′

absorption bands observed in SD PPI-MD samples where electrostatic interactions are not anticipated (Fig. 2c). On the other hand, the absorbance band and intensity remained unchanged in the physically mixed PPI-carrier samples (Fig. 2b & d), implying that there was no functional group interactions. In conclusion, FTIR results demonstrated that solid dispersion-based spray-drying processing modified the functional groups of PPI through hydrogen bonding and/or electrostatic interaction with amorphous matrix carriers.

In order to further understand the secondary structure changes of PPI-carrier upon solid dispersion-based spray-drying, the obtained spectra were analyzed by second derivative in the amide I band region (1700–1600 cm^{-1}; Fig. 3). The heavily overlapped amide I band region (1700–1600 cm^{-1}; Fig. 3) of protein typically reflects the characteristics of α-helix and β-sheet secondary structures presented. Second derivative of the spectra, a technique to increase the resolution of IR spectra, was applied to identify the secondary structures and assess their relative amounts in all the samples.

In parent PPI, the predominant secondary structure is β-sheet, which is commonly found in the interior of the folded hydrophobic sites of proteins (Wang, Li, Jiang, Qi, & Zhou, 2014). A significant decrease of α-helix and β-sheet was noticed in all spray-dried PPI-carrier samples as PPI to carrier ratio declined from 90:10 to 60:40 (Fig. 3a & c). Conversely, there was only a slight reduction of β-sheet in physically mixed PPI-carrier samples and no α-helix structure changes were observed (Fig. 3b & d). In general, the loss of highly ordered secondary structures denotes the weakening of hydrogen bond strength and hydrophobic interactions of peptide groups in protein. The decreased hydrogen bond strength in a partially unfolded PPI peptide group may facilitate competing hydrogen bonding to hydrophilic amorphous matrix carriers (GA and MD), leading to the formation of a permanently disordered PPI and the enhancement of total hydrophilicity after spray-drying (Soltanizadeh et al., 2014). Likewise, such interaction was reported in the complex formation of pea globulin and acacia gum through hydrogen bonding between protein β-sheet-rich regions and polysaccharides (Ducel, Pouliquen, Richard, & Boury, 2008). Meanwhile, greater secondary structure loss was also seen in PPI-GA than in PPI-MD after spray-drying, which may be contributed by the stronger electrostatic interactions between PPI and GA, as revealed by FTIR.

3.3. X-ray diffraction (XRD)

XRD analysis is a technique to provide structural information of molecules, such as amorphous, semi-crystalline, and crystalline structures (Gulao, de Souza, da Silva, Coimbra, & Garcia-Rojas, 2014). The XRD spectrum of spray-dried (SD) and physically mixed (PM) samples of PPI-carrier (GA or MD) is presented in Fig. 4.

Two broad diffraction peaks, presenting at 8.9° and 19.5° (2θ) in the XRD spectrum of PPI, were identified as the characteristic diffraction peaks of PPI (Fig. 4a), indicating a primarily amorphous structure with a minimum crystallinity (Borrmann, Pierucci, Leite, & Leao, 2013; Nambiar, Sellamuthu, & Perumal, 2017). Interestingly, these two identified diffraction peaks were very close to the characteristic peaks of whey protein isolate (8.83° and 19.66°) (Gonzalez-Martinez et al., 2017) and soy protein isolate (9° and 19°) (Wang, Xiao, et al., 2014), but appreciably different from lentil protein isolate (10° and 24°) (Joshi et al., 2011). In terms of carrier, the XRD profiles of GA and MD showed a single characteristic broad peak (Fig. 4a & c), similar to the previous observations (Cozic, Picton, Garda, Marlhoux, & Le Cerf, 2009;

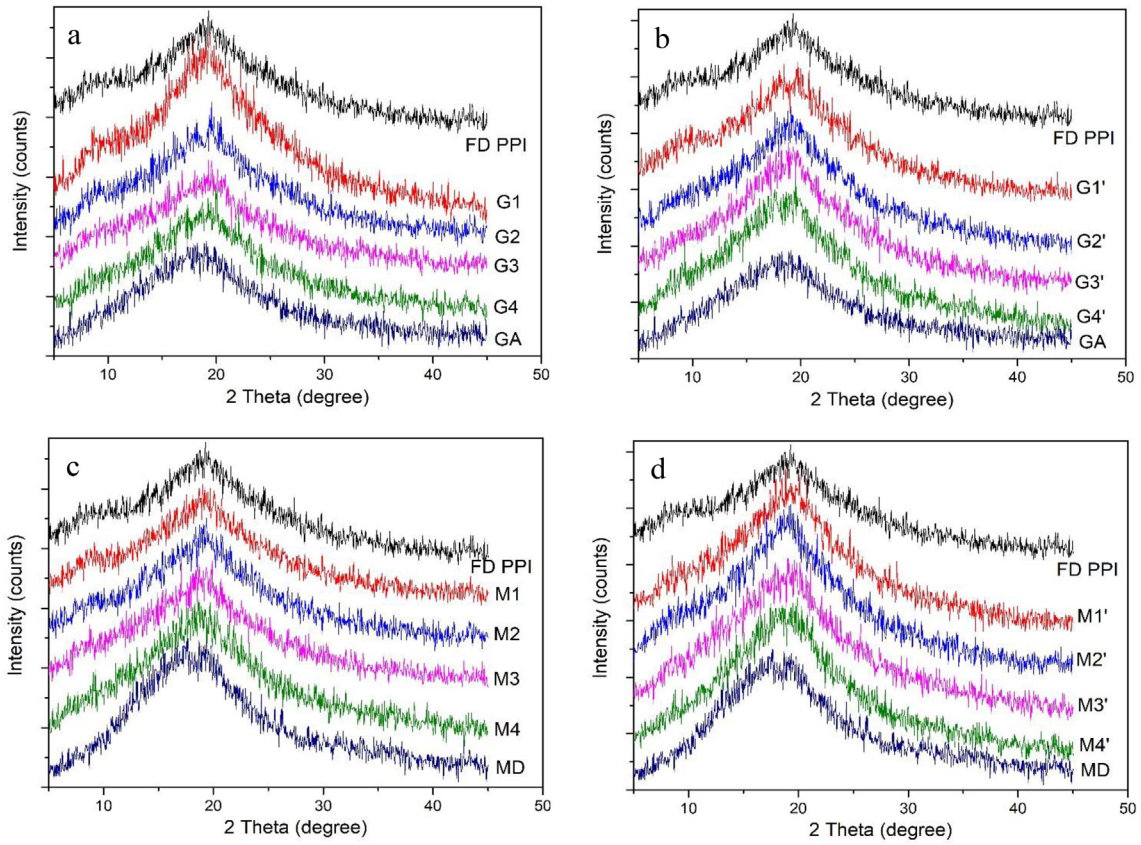

Fig.4. X-ray diffraction pattern of controls and spray-dried samples. The patterns of (a) FD-PPI, GA, and spray-dried G1-G4, (b) physically mixed G1′-G4′, (c) MD, and (d) spray-dried M1-M4 and physically mixed M1′-M4′.

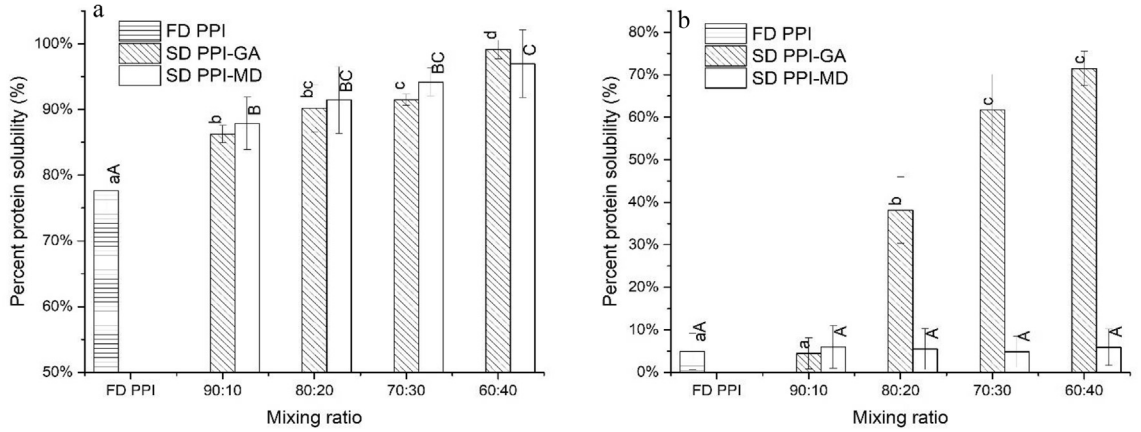

Fig. 5. The dependence of percent protein solubility (PPS) on PPI-GA or PPI-MD mixing ratios tested at (a) pH 7 and (b) 4.5. The different lowercase and uppercase letters indicate significant differences between different ratios of PPI-GA and PPI-MD, respectively ($p < .05$). FD represents freeze-dried samples, and SD represents spray-dried samples.

Gonzalez-Martinez et al., 2017), indicating their amorphous nature.

For SD PPI-carrier powder (PPI-GA or PPI-MD), both G1 and M1 showed two broad peaks, which is close to the pattern of parent PPI (Figs. 4a & 3c). The characteristic peak of PPI at around 19.5° became broader in the spray-dried samples as the content of carriers increased from 10% to 40% w/w (Fig. 4a & c), suggesting that the addition of carrier transforms PPI to a greater amorphous character after spray-drying. In contrast, no such phenomenon was observed in PM samples (Fig. 4b & d). Interesting, the peak intensity of PPI at 8.9° was gradually reduced as the content of carriers increased from 10% to 30%, while this peak disappeared when 40% carriers was incorporated in PPI after spray-drying (Fig. 4a & c). A similar trend also occurred in PM samples (Fig. 4b & d). We attributed this to the weak characteristic peak of PPI at 8.9° so that it becomes undetectable as the content of PPI dropped to 60%, regardless of the systems. This phenomenon was also reported in solid dispersion samples of Rebaudioside D (Reb D) and potassium sorbate (Pang et al., 2015). The majority of the characteristic peak of Reb D disappeared when 50% of potassium sorbate was incorporated into Reb D by physical mixing.

The XRD results, along with SEM and FTIR findings, allow us to depict the pattern of structural changes between PPI and amorphous carrier (GA or MD) during spray-drying. Solid dispersion of PPI with amorphous carrier by spray-drying could first cause the exposure of hydrophobic sites of PPI. Instead of forming bigger aggregates via

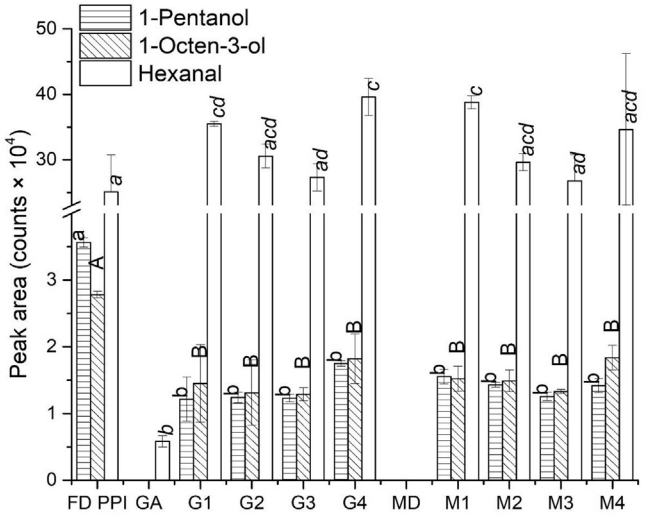

Fig. 6. Beany flavour and lipid oxidation-associated volatile compounds in controls and spray-dried samples. The different lowercase, uppercase, and italic lowercase letters indicate significant differences of 1-pentanol, 1-octen-3-ol, and hexanal, respectively, between all samples ($p < .05$).

hydrophobic interaction between unfolded secondary structures of pure PPI, the inclusion of amorphous GA or MD may form a complex with unfolded PPI through hydrogen bonding and/or electrostatic interaction. In consequence, the loss of the inherent ordered structures of PPI will facilitate the formation of powders of greater amorphous state and smaller particle size.

3.4. Percent protein solubility

Solubility is an important prerequisite for proteins to be used as effective functional ingredients in a number of high moisture foods, such as emulsions, foams, and beverages. In order to understand how the solid dispersion-based spray-drying technique could impact the solubility of PPI, the percent protein solubility (PPS) of SD PPI-carrier samples at pH 4.5 and 7 was measured and compared to that of FD PPI (Fig. 5).

At neutral pH (pH 7), the PPS of FD PPI was 76% (Fig. 5a), again indicating its poor solubility. The presence of carrier (GA or MD) greatly boosted PPS at neutral pH across all tested mixing ratios, and PPS reached 98.7% and 95.5%, for PPI-GA and PPI-MD, respectively, at the mixing ratio of 60:40 (w/w). This can be explained by the gradually increased surface to volume ratio (Fig. 1), the decreased ordered secondary structures and the increased total hydrophilicity (Fig. 3a & c), and the slightly reduced crystallinity of PPI (Fig. 4a & c) when PPI was dispersed with carriers via hydrogen bonding and electrostatic interaction after spray-drying (Fig. 2a & c). Regarding the different carriers (GA and MD), there was no significant difference in terms of PPS at all mixing ratios under neutral pH ($p > .05$)

However, a marked difference of PPS was noticed between two carriers (GA and MD) at pH 4.5 (Fig. 5b). The results clearly showed that carrier had remarkable effects on PPI solubility and aggregation under acidic pH conditions. The PPS of FD PPI was extremely low (4.4%), which is not surprising since the environmental pH is so close to its isoelectric point (pH ~4.6) (Lan et al., 2018). There was no significant difference in PPS between FD PPI and all mixing ratios of SD PPI-MD, indicating that solid dispersion of PPI with MD was unable to reduce the aggregation of protein at pH close its isoelectric point (Fig. 5b). In other words, the positive attributes (e.g., increased S/V ratio and decreased crystallinity) of SD PPI-MD derived from the solid dispersion-based spray-drying technique were completely shielded by the extensive protein aggregation around its isoelectric point. Unlike SD PPI-MD, PPS of SD PPI-GA was greatly improved and presented a GA concentration-dependence manner (Fig. 5b). For example, no significant difference of PPS was observed between SD PPI-GA at a ratio of 90:10 w/w and FD PPI ($p > .05$). In contrast, the PPS of SD PPI–GA (60:40) and FD PPI were 71.47% and 5.91%, respectively, at pH 4.5. Such phenomena can again be attributed to the unfolding of the highly ordered secondary structure of PPI during spray-drying. Instead of forming agglomeration by the re-aggregation of dissociated protein, as can be seen in other plant protein after spray-drying, PPI remains in amorphous form via solid dispersion with GA which could increase the hydrophilicity of mixture and surface area/volume ratio (Felix da Silva, Ahrné, Larsen, Hougaard, & Ipsen, 2018; Pang et al., 2015). Therefore, more interaction sites between spray-dried sample materials and water are anticipated, resulting in the increase of solubility and dispersion rate of PPI. Additionally, GA is an anionic biopolymer containing carboxyl groups with a pKa of 2.2 (Chanamai & McClements, 2002). The carboxyl groups are deprotonated at pH 4.5 which could prepare sufficient negative charges at higher GA concentrations. The electrostatic interactions between negatively charged GA and positively charged protein patches, as evidenced by FTIR results (Fig. 2a), may prevent protein aggregation by forming soluble complexes.

3.5. Effect of carrier type and PPI to carrier ratio on off-flavour compounds in pea protein isolate

Paramount barriers against human consumption of pea protein are its beany and rancid flavours. In general, around 10–20 core volatile compounds have been identified in pea protein which are responsible for off-flavour (Murat, Bard, Dhalleine, & Cayot, 2013). In this section, three common volatile compounds, namely, 1-pentanol, 1-octen-3-ol and hexanal, were selected as markers of off-flavour compounds in PPI and quantified using SPME-GC–MS. Among them, 1-pentanol and 1-octen-3-ol, lipoxygenase catalyzed oxidation products of unsaturated fatty acids, are generally considered as compounds responsible for beany flavor, while hexanal can be formed by lipoxygenase-catalyzed oxidation and/or free radical chain-initiated lipid oxidation in pea protein (Oomah, Razafindrainibe, & Drover, 2014; Wang, Dou, Macura, Durance, & Nakai, 1997). Due to the fact that no beany flavour can be detected by the sensory panel when protein contains any concentrations of hexanal (Vara-Ubol, Chambers, & Chambers, 2004; Wang, Jiang, & Zhou, 2013), hexanal is considered as an indicator of free radical chain-initiated oxidation products in this study. The effects of carrier type (GA and MD) and PPI to carrier mixing ratio (90:10–60:40) on the concentration of three selected volatile compounds are shown in Fig. 6.

Notably, high amounts of both beany flavour compounds (1-pentanol and 1-octen-3-ol) and rancid marker hexanal were detected in FD PPI, indicating the accumulation of beany and rancid flavour compounds via strong lipid oxidation activities (Fig. 6). Similar results were reported on field green pea cultivars under different storage conditions (Azarnia et al., 2011). Meanwhile, none of these off-flavour compounds were detected in MD; whereas no beany flavour compounds (1-pentanol and 1-octen-3-ol), but a trace of hexanal were detected in GA. The appearance of oxidation product hexanal in GA is not surprising since a small amount of fatty acid (0.009–0.05 mol/mol GA) is anchored with GA which could undergo lipid oxidation (Yadav, Igartuburu, Yan, & Nothnagel, 2007).

After the formation of solid dispersion via spray-drying, there was an appreciable decrease of beany flavours in SD PPI-carriers as compared to FD PPI (Fig. 6). In particular, the contents of 1-pentanol and 1-octen-3-ol declined 3-fold and 2-fold, respectively, upon the formation of SD PPI-carrier samples. Interestingly, there was no significant difference ($p > .05$) in the contents of two beany flavour volatiles in PPI solid dispersed with both carriers, regardless of mixing ratios (Fig. 6). The results suggested that a small amount of carriers (e.g., 10%) was enough to mitigate the generated beany flavour compounds in PPI. In general, plant protein has a strong affinity for off-flavour volatile compounds which makes it extremely difficult to remove. The release of

beany flavour compounds from PPI via the solid dispersion-based spray-drying technique can be explained by the reduction of intermolecular hydrophobic interactions of peptide groups, resulting in the decrease of bond strength with hydrophobic volatiles. As such, one would expect that the released beany flavour compounds would be stripped off during the high temperature (160 °C) spray-drying processing. In addition, high temperature of spray-drying processing (160 °C) might facilitate the inactivation of lipoxygenase that stops the production of beany flavour volatiles. This is supported by the finding that the activity of lipoxygenase was inhibited using a microwave at 116 °C (Jiang et al., 2016).

Meanwhile, a negative impact on oxidized flavour (hexanal) mitigation was also observed. The total area counts of hexanal were dramatically increased in all SD PPI-carrier samples as compared to FD PPI. Two reasons might account for this. First, plant protein is proven to have a higher binding affinity with aldehydo flavour compounds (e.g., hexanal) than have alcohols (e.g., 1-pentanol and 1-octen-3-ol) (Wang, Zhao, Qiu, & Sun, 2018). This allows beany flavour alcohols to be easily released upon the unfolding of PPI, and thence stripped off during spray-drying. Second, free radical-initiated lipid oxidation will be accelerated during high temperature spray-drying processing, giving rise to the production of hexanal (Jiang et al., 2016; Park, Stout, & Drake, 2016). For instance, Jiang et al. (2016) reported that hexanal became a major volatile compound after microwave treatment for 1.5 min due to the promotion of a non-enzymatic lipid oxidation. In this case, special care or antioxidant strategies should be developed to both mitigate beany flavour and inhibit the newly formed rancid flavour when applying solid dispersion-based spray-drying processing.

4. Conclusions

Solid dispersion-based spray-drying would be a promising technology to broaden the application of pea protein isolate. The results obtained from SEM, FTIR, and XRD indicated structural differences between spray-dried and physically mixed PPI-carrier samples. The carrier type (gum arabic and maltodextrin) and PPI to carrier ratio (90:10, 80:20, 70:30, and 60:40) strongly influenced the solubility of pea protein isolate. The enhanced solubility of spray-dried PPI-carrier samples at pH 4.5 can be explained by the increase of surface area/volume ratio, and the formation of hydrogen bonds and electrostatic interaction between PPI and carrier. The contents of 1-pentanol and 1-octen-3-ol, beany flavour markers, declined 3-fold and 2-fold, respectively, as compared to parent PPI, by virtue of the unfolding of the secondary structure of PPI with the assistance of amorphous matrix carrier (gum arabic and maltodextrin). However, accelerated lipid oxidation can still occur during high temperature spray-drying. Our study has provided a better understanding of the role of gum arabic and maltodextrin in the improved functionalities of pea protein isolate via solid dispersion-based spray-drying processing, which can further facilitate the application of pea protein in food and beverages.

Declaration of interest

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be affecting the objectivity of this article.

Acknowledgements

This work was supported by USDA National Institute of Food and Agriculture (Hatch ND1594) and Northern Pulse Growers Association (FAR0027461). A scholarship for Mr. Yang Lan is provided by the China Scholarship Council (CSC).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.11.074.

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